Polymeric proteins and their association with grain yield in hard red spring wheat lines

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Abstract Development of high yielding wheat (*Triticum aestivum* L.) varieties with acceptable end-use quality is a major focus in breeding programs worldwide. Variations in molecular weight (Mw) distribution of endosperm proteins are known to influence end-use quality traits. This paper reports the relationship of the size-exclusion high performance liquid chromatography (SE-HPLC) profile of endosperm proteins with grain yield. Flour samples were previously analyzed for Mw distribution of sodium-dodecyl sulfate (SDS) extractable and unextractable proteins using the SE-HPLC protocol. Correlations were calculated between grain yield and HPLC absorbance data obtained at 0.01-min retention time intervals. Although both SDS-extractable and unextractable proteins had positive correlations with grain protein content, only SDS-unextractable very high Mw polymeric proteins (UVHP) had no negative association with grain yield, while SDS-extractable fractions rich in low Mw polymeric proteins had a negative correlation ($r = -0.41$) with grain yield. These results suggest that in an effort to increase grain yield, breeding programs should target grain yield and also increase levels of UVHP and decrease SDS-extractable polymeric proteins, thereby, maintaining acceptable bread-making quality.

Keywords SDS-extractable polymeric proteins · SDS-unextractable polymeric proteins · Wheat bread-making quality · Grain yield · Grain protein content

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Introduction

Development of high grain yield varieties with acceptable end-use quality is a major focus in wheat breeding programs worldwide. Because whole grain protein content is very important for wheat end-use quality, several researchers have studied the genetic basis of grain protein content on a whole kernel basis (Prasad et al. 1999; Campbell et al. 2001; McCartney et al. 2006). Although it was possible to develop wheat cultivars with high grain yield and high grain protein content (Stuber et al. 1962; Johnson et al. 1973), several wheat researchers have shown that these two

traits are often negatively associated (Malloch and Newton [1934](#); Terman et al. [1969](#); Löffler and Busch [1982](#); Groos et al. [2003](#); Tsilo et al. [2010a](#)); thereby impeding simultaneous improvement of both traits.

The bread-making quality of hard spring and winter wheats is directly related to gluten (Finney and Yamazaki [1967](#)). The wheat gluten proteins or endosperm proteins have extensively been analyzed by size-exclusion high performance liquid chromatography (SE-HPLC) since the initial work by Bietz ([1984](#)). Using SE-HPLC, wheat proteins could be separated based on their molecular size/weight, in an order of large to small proteins. Several studies have reported significant associations between the variation in molecular weight (Mw) distribution of endosperm proteins and end-use quality of different classes of wheat (Bangur et al. [1997](#); Borneo and Khan [1999](#); Suchy et al. [2003](#); Ohm et al. [2006](#)). For bread-making quality, it was initially reported by Gupta et al. ([1993](#)) that the percentage of SDS-unextractable polymeric protein (UPP) in total polymeric proteins strongly affected dough strength parameters positively. Recently, Tsilo et al. ([2010b](#)) reported the associations of dough mixing strength and bread-making properties with specific protein fractions or components of the SDS-extractable and unextractable polymeric proteins, as determined by SE-HPLC. In that study, the authors found that the SDS-unextractable very high Mw polymeric proteins (UVHP), eluted mainly at the front section of SE-HPLC chromatogram, had much stronger positive relationship with dough mixing strength and bread loaf volume compared with other UPP fractions. The authors also reported that the SDS-extractable polymeric proteins (EPP) had significant negative correlations with dough mixing strength. Thus, it was concluded that it might be better to breed for higher UVHP and very low EPP in breeding for improved bread-making quality.

Several researchers have studied the relationship of grain yield and wheat protein based on whole kernel protein content as mentioned before. However, considering that individual endosperm protein fractions are known to affect bread-making quality attributes differently, their relationship with grain yield has not been studied. The purpose of this research is to describe the relationship of grain yield and protein in more detail, particularly in regard to how specific protein fractions of SE-HPLC relate to grain yield.

Materials and methods

Plant materials and quality analysis

A mapping population of 139 $F_{6:8}$ recombinant inbred lines (RILs) used in this study was developed from the cross between the two hard red spring wheat breeding lines MN98550 and MN99394 (Tsilo et al. [2011](#)). MN98550 originated from a cross of 'BacUp' (Busch et al. [1998](#)) and 'McVey' (Busch et al. [2001](#)), while MN99394 originated from the cross of SD3236/SBF0402. Although this germplasm produces high grain yield and is adapted to the upper midwest region of the United States, the primary reason of developing this mapping population was to study end-use quality traits, and the parental lines were chosen because they had different quality attributes. MN98550 carries high molecular weight glutenin subunits or alleles of Ax-null, Bx7+By8, and Dx5+Dy10 at the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci, respectively, whereas MN99394 carries Ax2* and Dx2+Dy12. The field trials were grown at three Minnesota locations in 2005 and 2006 to produce grain for quality analyses. The 2005 grain had very low test weight due to rust damage, and this population segregated for resistance to both leaf and stem rust diseases (Tsilo [2009](#)), and the grain was not included in the current analysis. As described previously by Tsilo et al. ([2010a](#)), each experimental line was planted using a yield trial plot size of 2.6 m^2 with seven rows, and plots were laid out in a randomized complete block design. Field trials included 139 RILs, two parents, and the three check varieties 'Alsen' (Frohberg et al. [2006](#)), 'Verde' (Busch et al. [1996](#)) and 'Oklee' (Anderson et al. [2005](#)). Grain yield, yield components, and other agronomic traits that were evaluated in this population were previously described (Tsilo et al. [2010a](#)). For RILs, two replicates were grown in all trials, and the grain samples from these replicates were bulked to provide grain for quality analysis. Checks had eight replicates and grain samples were bulked to produce four replicates per check. Grain samples harvested from field trials were submitted for quality analyses at the USDA-ARS Wheat Quality Laboratory, Fargo, North Dakota. Whole grain protein was determined by near infrared transmittance with an Infratec 1225 Grain Analyzer (Foss North America, Silver Springs, MD). Grain was tempered to 16.5 % moisture content and milled using Quadrumat Senior Break and

Reduction grinding heads (C.W. Brabender Instrument Inc., South Hackensack, NJ).

Protein characterization

The SE-HPLC assays and analyses followed protocols previously described by Ohm et al. (2008). Briefly, the SDS extractable and unextractable protein fractions were separated based on the protocols of Batey et al. (1991) and Gupta et al. (1993). Solutes were detected at the absorbance of 214 nm using Agilent 1200 Photodiode Array Detector (Agilent technologies, Santa Clara, CA). Absorbance data were interpolated to 0.002-min intervals and the absorbance area (AA) was calculated by mean absorbance × time interval of 0.002 min. Means of replicated samples were calculated for all protein fractions. The AA values for total protein were mathematically estimated by adding AA values of extractable and unextractable protein fractions (Fig. 1). Linear correlation coefficients (r) were calculated between both mean values of AA and quality parameters and shown as a continuous spectrum over retention time for each 0.01-min retention interval. The sum of AA for each retention time interval of 0.01 min was used for correlation analysis between 3.6 and 7.7 min of run time. The AA values of major protein fractions (Fr1–Fr3), as shown in Fig. 1, represent fractions of polymeric proteins:

Extractable Polymeric Proteins (EPP)
   = Fr1 + Fr2 + Fr3 of extractable proteins

Fig. 1 Typical size exclusion HPLC chromatograms of sodium dodecyl sulfate (*SDS*) extractable and unextractable proteins, and total protein, showing protein fractions (*Fr*) at different retention times

Unextractable Polymeric Proteins (UPP)
   = Fr1 + Fr2 + Fr3 of unextractable proteins

Unextractable Very High Mw Polymeric Proteins
   × (UVHP) = Fr1 of unextractable proteins

These polymeric protein fractions were identified as unique protein fractions associated with dough-mixing strength and bread-making properties (Gupta et al. 1993; Zhang et al. 2008; Tsilo et al. 2010b). The SE-HPLC AA and area % (A %) values represent quantity of protein fractions in the flour and protein, respectively.

Statistical analysis

Analysis of variance (ANOVA) was conducted for all major protein fractions over all environments using SAS statistical software package version 9.1 (SAS Institute, Cary, NC). Because samples were bulked over replications within environments, the main effects of RILs and environment were tested for significance using the genotype × environment mean square as an error term. The genotype × environment interaction was tested for significance using the error mean square estimated from the check genotypes that were replicated within environments, as described in an augmented design by Federer (1961). The Fisher's least significant difference ($LSD_{0.05}$) calculated from the error mean square was used to test the difference between the means of two parental types. The results of ANOVA were used to obtain the broad-sense heritability estimates (h_B^2):

$$h_B^2 = \frac{\sigma_g^2}{\left[\sigma_g^2 + \left(\sigma_{ge}^2/e\right) + \left(\sigma_e^2/re\right)\right]} \text{ or } 1 - \frac{MS_{ge}}{MS_g}$$

where MS_g and MS_{ge} represent the genotype and genotype by environment mean squares, respectively, σ_g^2 is the genotypic variance $= (MS_g - MS_{ge})/(re)$, σ_{ge}^2 is the genotype × environment interaction variance $= (MS_{ge} - MS_e)/r$, and σ_e^2 is the error variance $= MS_e$, r is number of replications, and e is number of environments. The phenotypic distributions of traits based on the mean of three environments were tested for normality using the Shapiro–Wilk statistic (Shapiro and Wilk 1965). The relationships of all major protein fractions were assessed by Pearson correlation coefficients using PROC CORR of SAS.
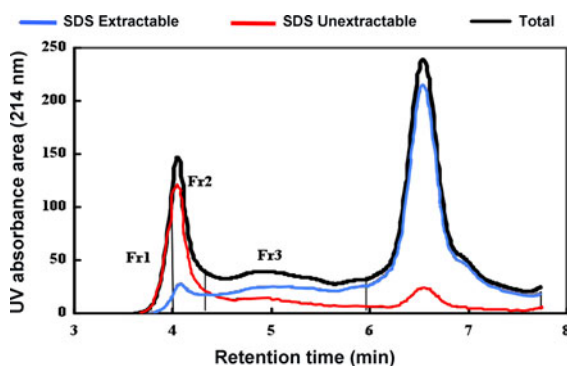
Results

Phenotypic variation and correlations involving endosperm polymeric proteins

Table 1 shows mean, minimum and maximum trait values for the AA of protein fractions (Fr1–Fr3, as shown in the size exclusion-HPLC chromatogram in Fig. 1) in three locations and their average. For example, the EPP, which are proteins eluted at the Fr1, Fr2, and Fr3 sections of the chromatogram, showed a mean of 4,615 and a range of 3,757 and 5,441 for the trait values averaged across environments. Even though both parents did not differ significantly in UPP and UPP/EPP ratio, MN98550 had significantly higher EPP, UVHP and UVHP/EPP ratio than MN99394. Transgressive segregation was observed in all major protein fractions with some RILs showing trait values beyond the parental range at the $LSD_{0.05}$, meaning that parents may differ at several loci controlling these protein fractions and both parents contributed favorable and unfavorable alleles. The effects of genotype, environment and their interactions were highly significant ($P \leq 0.001$) on all major protein fractions (Table 2), and their mean squares are shown on Table 2 for the purpose of estimating broad-sense heritability. Heritability estimates ranged from 0.63 to 0.78.

Correlations among all major endosperm protein fractions of SE-HPLC were highly significant ($P \leq 0.001$) with the exception of AA values of UPP and EPP (Table 3). Correlations were stronger with the A % than the AA values of protein fractions. For example, the EPP A % had significantly negative correlations with UPP and UVHP, including the ratios of UPP to EPP.

Relationship of SE-HPLC of endosperm proteins with grain yield and protein content

The relationships of SE-HPLC AA values of endosperm proteins with grain yield and grain protein content are shown as a continuous spectrum of correlation coefficients on the SE-HPLC chromatogram (Fig. 2a–d). These spectra of correlation coefficients display distinct statistical relationships between grain yield and protein fractions separated by SE-HPLC. As expected, all protein fractions of both SDS-extractable and unextractable proteins, at any given

retention time along 3.5 to 7.5 min, were positively correlated with grain protein content (Fig. 2a, c). As shown in Fig. 1, when the AA values of both SDS-extractable and unextractable protein fractions were added to mathematically estimate the total protein, their total values were significantly correlated with flour protein content ($r = 0.93$, $P \leq 0.001$) and grain protein content ($r = 0.90$, $P \leq 0.001$). Of all protein fractions along the chromatogram of SE-HPLC, SDS-unextractable proteins showed variable (insignificant to significant) correlations with grain yield (Fig. 2b). Of these SDS-unextractable proteins, mainly protein fractions that were eluted within the front section of the chromatogram, which are referred to as UVHP, had no significant negative association with grain yield (Fig. 2b). Furthermore, proteins eluted in this front section have no negative relationship with test weight, 1,000-kernel weight, percentage of large kernels, single-kernel hardness, flour yield, and heading date (Fig. 3a–f). Although these proteins had a significant positive relationship with test weight and heading date (Fig. 3a, f), they have somehow a favorable negative relationship with plant height (Fig. 3g), suggesting that an increase in UVHP will not have any adverse effect on the production and yield. Of all protein fractions along the chromatogram of SE-HPLC, SDS-extractable proteins also showed variable (significant to insignificant) correlations with grain yield (Fig. 2d). The negative correlations along the chromatogram reached a peak within 5–6 min with a maximum correlation of −0.41 between SDS-extractable proteins and grain yield (Fig. 2d), suggesting that the EPP that were eluted within the Fr3 section of the SE-HPLC chromatogram have a strong undesirable relationship with grain yield. SDS-extractable proteins that were eluted within the Fr4 section of the chromatogram (6–7 min) showed low or no correlation with grain yield (Fig. 2d).

Discussion

Phenotypic variation and correlations involving endosperm proteins

Recently, a negative correlation ($r = -0.41$, $P \leq 0.001$) was reported between grain yield and grain protein content (Tsilo et al. 2010a). Previous studies have shown that the negative correlations between

Table 1 Mean values, standard deviations, and range of endosperm proteins in a recombinant-inbred population evaluated in three environments in 2006

Trait[a]	Environment	RIL population ($n = 139$)				LSD$_{0.05}$	Parental lines[c]	
		Mean	Min	Max	Normality[b]		MN99394	MN98550
EPP (AA)	All	4615	3757	5441	$W = 0.99$	142	4543b	4711a
	Crookston	4203	3394	4995	(0.178)		4145	4396
	Morris	5005	3992	6403			4906	4917
	St. Paul	4636	3704	5780			4577	4820
EPP (A %)	All	20.67	17.62	23.23	$W = 1.00$	0.48	19.78b	20.42a
	Crookston	19.92	17.13	22.75	(0.955)		18.92	19.87
	Morris	21.03	17.29	24.71			19.97	20.31
	St. Paul	21.05	17.93	26.73			20.44	21.10
UVHP (AA)	All	951	599	1277	$W = 1.00$	53	948b	1045a
	Crookston	904	604	1313	(0.951)		928	1006
	Morris	1055	665	1575			1072	1147
	St. Paul	895	286	1259			846	980
UVHP (A %)	All	4.26	2.74	5.52	$W = 1.00$	0.19	4.13b	4.52a
	Crookston	4.29	2.69	5.67	(0.873)		4.24	4.55
	Morris	4.44	2.89	6.45			4.37	4.73
	St. Paul	4.10	1.65	5.52			3.77	4.28
UVHP/	All	0.21	0.12	0.28	$W = 0.99$	0.01	0.209b	0.222a
EPP	Crookston	0.22	0.13	0.31	(0.429)		0.224	0.229
	Morris	0.21	0.12	0.31			0.219	0.233
	St. Paul	0.19	0.06	0.29			0.185	0.203
UPP (AA)	All	4921	3085	6216	$W = 0.97$	131	5084a	5075a
	Crookston	4813	3702	6186	(0.002)		4968	4971
	Morris	5144	3660	7183			5391	5335
	St. Paul	4805	1761	5977			4893	4919
UPP (A %)	All	22.1	14.5	25.7	$W = 0.97$	0.43	22.15a	22.00a
	Crookston	22.8	17.7	26.7	(0.003)		22.70	22.44
	Morris	21.7	15.1	25.9			21.91	22.03
	St. Paul	21.8	10.1	26.0			21.84	21.51
UPP/	All	1.08	0.64	1.38	$W = 0.987$	0.05	1.124a	1.080a
EPP	Crookston	1.15	0.82	1.50	(0.230)		1.201	1.131
	Morris	1.03	0.65	1.48			1.102	1.088
	St. Paul	1.04	0.38	1.37			1.069	1.020

[a] *EPP* HPLC absorbance area of SDS-extractable polymeric proteins (Fr1 + Fr2 + Fr3 of Fig. 1), *UPP* HPLC absorbance area of SDS-unextractable polymeric proteins (Fr1 + Fr2 + Fr3 of Fig. 1), *UVHP* HPLC absorbance area of SDS-unextractable very high molecular weight polymeric proteins (Fr1 of Fig. 1), *AA* total absorbance area of protein fractions in the flour, *A %* percentage of AA in total proteins

[b] The phenotypic distributions based on the mean of three environments were tested for normality using the Shapiro–Wilk statistic (Shapiro and Wilk 1965); *P* values in parenthesis

[c] Means of parents are significantly different if followed by different letters (LSD$_{0.05}$)

these two traits ranged from moderate to strongly significant values, for example, $r = -0.40$ (Groos et al. 2003), $r = -0.48$ (Löffler and Busch 1982), $r = -0.92$ to -0.97 (Terman et al. 1969). In the current study, we did however, analyze the relationship between these two traits, taking into consideration that

Table 2 Mean squares and heritabilities for endosperm protein fractions of a recombinant inbred line population ($n = 139$) evaluated in three environments in 2006

Endosperm protein fractions[a]	Mean square				
	Genotype	Environment	G × E[b]	Error[c]	h_B^{2d}
EPP (AA)	3.3×10^5***	2.2×10^7***	1.1×10^5***	49667	0.68
EPP (A %)	3.53***	58.14***	1.12***	0.56	0.68
UVHP (AA)	41386***	1.1×10^6***	15123***	6917	0.63
UVHP (A %)	0.79***	5.33***	0.27***	0.09	0.66
UVHP/EPP	0.003***	0.020**	7.96×10^{-4}***	3.53×10^{-4}	0.73
UPP (AA)	4.8×10^5***	5.2×10^6***	1.6×10^5***	41660	0.66
UPP (A %)	8.6***	56.7***	1.9***	0.45	0.78
UPP/EPP	0.041***	0.59***	0.011***	0.005	0.73

*** A significance at $P \leq 0.001$

[a] Trait explained in Table 1

[b] Genotype by environment interaction

[c] Error mean squares were estimated from the check genotypes that were replicated within environments, as described in an augmented design by Federer (1961)

[d] Broad-sense heritability on an entry-mean basis

Table 3 Phenotypic correlation coefficients among endosperm proteins based on trait values averaged across three environments in 2006

Traits	EPP (AA)	EPP (A %)	UVHP (AA)	UVHP (A %)	UVHP: EPP	UPP (AA)	UPP (A %)
Endosperm protein fractions[a]							
EPP (A %)	0.68***	1					
UVHP (AA)	−0.18*	−0.57***	1				
UVHP (A %)	−0.50***	−0.56***	0.90***	1			
UVHP/EPP	−0.62***	−0.77**	0.88***	0.96***	1		
UPP (AA)	−0.01[ns]	−0.46***	0.75***	0.59***	0.59***	1	
UPP (A %)	−0.49***	−0.45***	0.59***	0.73**	0.70***	0.77***	1
UPP/EPP	−0.67***	−0.79***	0.67***	0.77***	0.86***	0.74***	0.90***

*, **, and *** Significance at $P \leq 0.05$, 0.01, and 0.001, respectively; *ns* not significant at $P \leq 0.05$

[a] Trait explained in Table 1

grain proteins could be resolved in more detail based on their molecular weight and size using the SDS-extractable and unextractable protein fractions. Ohm et al. (2006) reported a predictive model (R^2 value of 0.984) of protein content based on SE-HPLC data and concluded that it could be used to explain quantitative variation in protein content. In the current study, the associations of grain yield with proteins were presented as a continuous correlation spectrum between grain yield and the SE-HPLC absorbance area values calculated at 0.01-min retention time intervals. Comparing several spectral correlations involving SDS-extractable versus unextractable proteins, although

they were both positively correlated with grain protein content, only SDS-extractable proteins eluted mainly in Fr3 had an undesirable relationship ($r = -0.41$) with grain yield, indicating that they accounted for the most part of negative association previously seen between grain yield and grain protein content. The SDS-extractable proteins that were eluted within the Fr4 section of the chromatogram (6–7 min) showed low or no correlation with grain yield. Recently, Tsilo et al. (2010b) reported that the percentage of SDS-extractable proteins eluted at the Fr1, Fr2 and Fr3 of the SE-HPLC chromatogram, referred to as SDS-extractable polymeric proteins (EPP A %), had undesirable
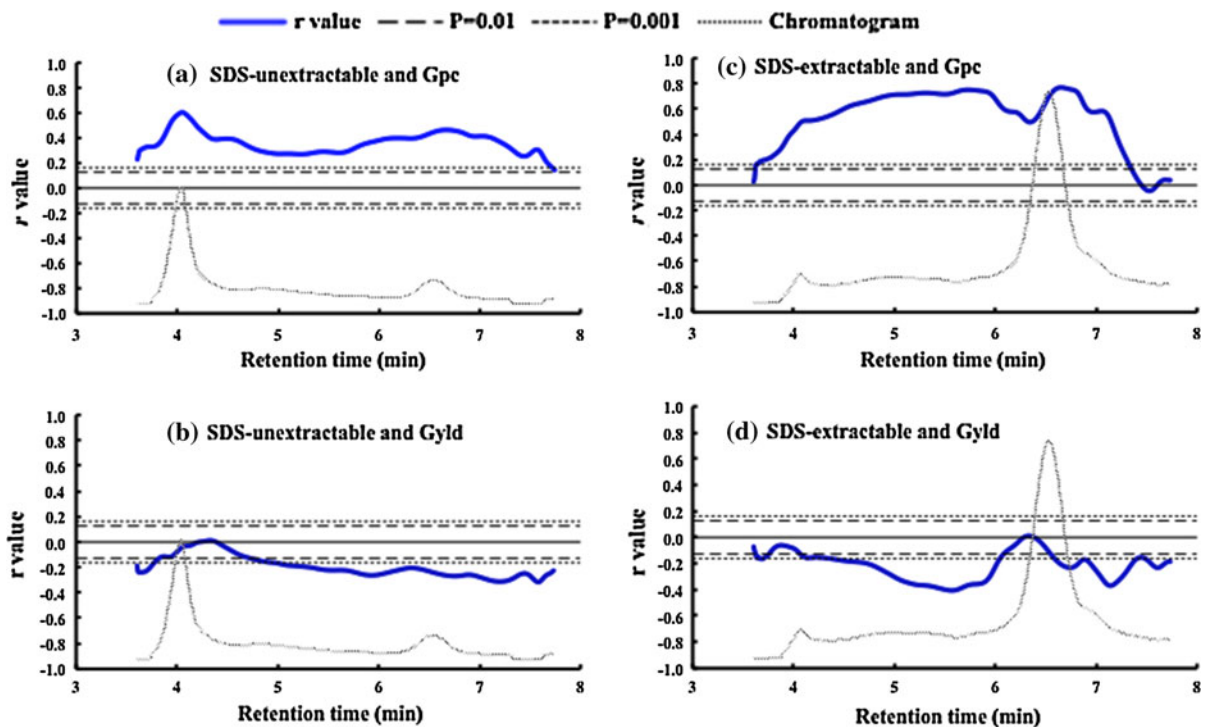
Fig. 2 Spectrum of correlation coefficients (*r*) between size-exclusion HPLC absorbance areas of proteins and grain properties: **a** SDS-unextractable proteins and grain protein content (*Gpc*), **b** SDS-unextractable proteins and grain yield (*Gyld*), **c** SDS-extractable proteins and Gpc, **d** SDS-extractable proteins and Gyld. The appearance of chromatogram only refers to the protein types involved in correlation spectrum, and were referenced as they appear in Fig. 1

relationships with dough rheological properties, bake mixing time, and bread loaf volume in this sample set. Based on EPP A %, EPP accounts for about 20 % of the total protein (Table 1). Based on the correlations presented in this study and those presented by Tsilo et al. (2010b), EPP seem evidently undesirable for both grain yield and bread-making properties, particularly those eluted within the Fr3 section of the SE-HPLC. Contrastingly, UVHP was observed to have significant and positive effects on flour protein content and bread-making quality (Tsilo et al. 2010b), and this protein fraction did not have any adverse effect on grain yield and its component. Based on UVHP A % and UPP A %, average values of UVHP and UPP accounted for about 4 and 22 % of the total protein, respectively (Table 1). However, the observed transgressive segregation (Table 1), with some RILs showing values beyond the parental ranges at LSD$_{0.05}$, provides evidence suggesting that improvement in % protein can be made by combining genes from different sources.

The results of this study could have important implication that breeders should breed for high grain yield and bread-making quality by making grain yield as the primary trait and also selecting for desirable protein fractions of SE-HPLC instead of relying entirely on whole grain protein content. Moreover, several studies have shown that specific protein fractions, as resolved by SE-HPLC, influenced end-use quality differently and should be targeted for selection (Singh et al. 1990; Huebner et al. 1997; Kuktaitie et al. 2004; Labuschagne et al. 2004; Békés et al. 2006; Ohm et al. 2008; Tsilo et al. 2010b). In a study of the genetic analysis of grain protein concentration and protein composition, Charmet et al. (2005) concluded that a balance between protein fractions and their aggregation status can be manipulated independently from grain protein content, thus offering breeders the opportunity to improve both grain yield and end-use quality despite the strong negative correlation between grain yield and protein content.

In the current study, we have shown that EPP of the Fr3 section of SE-HPLC chromatogram has a negative correlation of 0.41 with grain yield, while UVHP has no adverse effect on grain yield and some of the yield
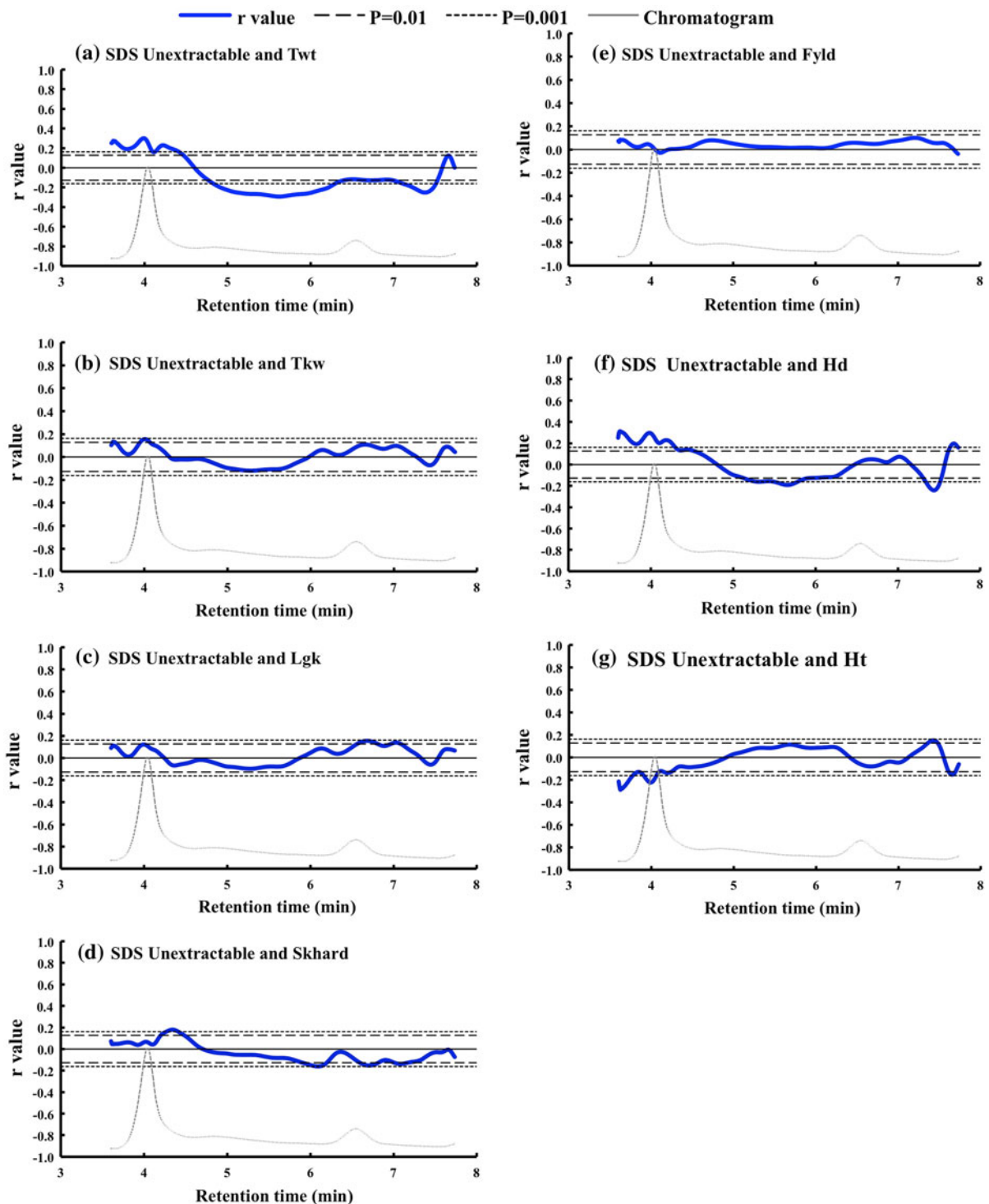
Fig. 3 Spectrum of correlation coefficients (*r*) between size-exclusion HPLC absorbance areas of SDS-unextractable proteins and **a** test weight (*Twt*), **b** 1,000-kernel weight (*Tkw*), **c** percentage of large kernels (*Lgk*), **d** single kernel hardness (*Skhard*), **e** flour yield (*Fyld*), **f** heading date (*Hd*), and **g** plant height (*Ht*)

components. Our hypothesis is that selection for increased quantity of UVHP will (a) increase grain protein content, and (b) improve bread-making quality without negatively impacting grain yield. This hypothesis is also supported by the conclusions of Charmet et al. (2005). However, before these results could have far-reaching implications, further research should be conducted to validate the relationship of grain yield with SE-HPLC of endosperm proteins in diverse genetic backgrounds of bread wheat and also in multi-year trials. Currently, we are conducting a study using a wide range of breeding material in a multi-year trial. Also, the recombinant inbred lines originating from this study were selected and used as parents in the breeding program. We hope that selection that is based on high grain yield and specific protein fractions will facilitate the development of high yielding wheat cultivars with acceptable quality.

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